2010 Research Grant Program Winning Abstract

Treg Cells in IL-2 Therapy

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Currently, high-dose (HD) IL-2 therapy for Stage IV melanoma can induce clinical responses in up to 15% of patients and it is the only current form of therapy capable of inducing long-lasting, durable remissions lasting for years in a small fraction (about 5%) of patients. At the MD Anderson Cancer Center, we treat over 100 patients annually with HD IL-2 therapy. However, although it has yielded these notable results in a fraction of patients, the vast majority of patients do not demonstrate appreciable clinical responses. These are attributed to a number of suppressive factors limiting anti-tumor T-cell activation and effector function. One of the most critical suppressive factors reducing the success of immunotherapy are CD4+CD25+Foxp+ T-regulatory (T-reg) cells both in the systemic circulation and in the tumor microenvironment. T-regs potently suppress both normal CD4+ and CD8+ T-cell activation and proliferation and in some cases can induce apoptosis of activated T-cells through cell-to-cell contact. Paradoxically, although HD IL-2 therapy activates and expands effector T cells and NK cells, recent work has clearly shown that it also increases the numbers of circulating T-regs. Similar findings have been made in ovarian and renal cancer patients treated with both low-dose and HD IL-2 regimens. Thus, T-reg expansion and/or activation during HD IL-2 therapy may be a key limiting factor in preventing anti-tumor lymphocyte activity and tumor eradication. We have found that the most highly suppressive T-reg subset expresses the costimulatory molecule ICOS and that high numbers of ICOS+ T-reg cells are found in metastatic melanoma lesions. Our data also shows that CD4+CD25hiFoxp3hiICOS+ T-reg cells massively increase in the blood of melanoma patients during the first cycle of HD IL-2, while the ICOS- T-reg subset only marginally changes. In addition, we found that melanoma cells in vivo express ICOS ligand (ICOS-L). In other experiments, we have isolated ICOS+ T-regs and found that they are significantly more sensitive to IL-2-induced STAT5 phoshosphorylation and AKT activation than ICOS- T-regs using BD™ Phosflow reagents. We hypothesize that CD4+CD25+Foxp3+ICOS+ T-cells expanded in melanoma patients during HD IL-2 therapy is a highly IL-2 sensitive potently suppressive T-reg subset inhibiting tumor-specific CD4+ and CD8+ CTL and that ICOS-L/B7H expression on melanoma cells can provide costimulatory signals facilitating the expansion and survival of this HD IL-2-induced T-reg subset. We also predict that either activating certain signaling pathways, such as Ox40, or blocking other signaling pathways (e.g., ICOS itself) can reverse the T-cell suppressive activity of this expanded T-reg subset. The specific aims of the project are:

Aim #1: To determine how the quantity and phenotype of ICOS+ T-reg cell population changes in relation to other lymphocytes in the peripheral blood of Stage IV melanoma patients during successive cycles of HD IL-2 therapy, and whether these changes correlate to clinical response.

Aim #2: To study the activation of different signaling pathways in response to HD IL-2 in isolated ICOS+ and ICOS- T-reg cells using BD Phosflow technology.

Aim #3: To study ways of blocking the survival and suppressive function of the expanded ICOS+ T-reg population isolated from patients on HD IL-2 therapy.

Our experiments will track the changes in ICOS+ T-reg cells as well as other lymphocyte subsets (T- and B- cells, NK cells, myeloid-derived suppressor cells, and dendritic cells) using multi-paramater FACS during HD IL-2 therapy in patients and correlate these changes to clinical responses. We will also characterize the phenotype and function of isolated IL-2-induced ICOS+ versus ICOS- T-regs from HD IL-2-treated patients and further test their responses to IL-2 and other cytokines, such as IL-15 and IL-7, using a larger panel of BD Phosflow reagents looking at the STAT, ERK, and AKT pathways. ICOS blockade or activation of TNF-R family members will be tested for their ability to reverse ICOS+ T-reg survival or function. Our results will form the basis for clinical trials testing ICOS+ T-reg blockade or TNF-R activation using humanized antibodies in patients as a synergistic therapy during HD IL-2 treatment. This ambitious project is ongoing and we have proof-of-concept data already obtained with a number of Becton Dickinson flow cytometry, BD Phosflow antibodies, and other related reagents. The project will continue to use a large volume of these antibodies over the next few years. In addition to helping develop novel approaches to augment the success if IL-2 therapy in cancer patients, these Becton Dickinson reagents will also aid in the generation of a comprehensive immunological profile in HD IL-2-treated patients to track additional lymphocyte subsets (e.g., NK cells, NK-like T-cells, and myeloid-derived suppressor cells) and how changes in these subsets relate to clinical response. Using this comprehensive immunological profiling we will track over 40 distinct lymphocyte subsets based on cell surface and intracellular (e.g., Perforin and Granzyme B) markers in order to help develop tools that predict which patients will respond to IL-2 and which patients are unresponsive. This will form part of a critical clinical decision-making tool that will facilitate better patient selection. This grant from Becton Dickinson will go a long way in helping us acquire the large number of different flow cytometry reagents needed for successful completion of this study.

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